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RESEARCH ARTICLE

Laboratory host range testing of *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) – a potential biological control agent of the invasive weed, Old World climbing fern, *Lygodium microphyllum* (Lygodiaceae)

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Old World climbing fern, *Lygodium microphyllum*, is a serious invasive weed in south Florida. Development of biological control is vital for sustainable management of *L. microphyllum*. *Neomusotima conspurcatalis* was discovered in Hong Kong in 1997 and was subsequently found causing feeding damage on *L. microphyllum* in much of its native range in Asia. Quarantine testing of *N. conspurcatalis* used 37 non-*Lygodium* fern species representative of New World genera of cultivated ferns and fern allies, one gymnosperm, three crop species, six *Lygodium* species, and the primary host *L. microphyllum*. No significant oviposition or feeding was observed on any of the 41 non-*Lygodium* species evaluated. Oviposition and feeding occurred on all *Lygodium* species, but amounts were low and usually significantly less than observed on *L. microphyllum*. The exception was *L. japonicum*, which was preferred as an oviposition host. *Neomusotima conspurcatalis* was only able to complete development on *L. japonicum* and *L. palmatum*, but survival on these species was only half that occurring on *L. microphyllum*. *Neomusotima conspurcatalis* is a *Lygodium* specialist. *Lygodium japonicum* is an invasive weed in the United States. *Lygodium palmatum* is restricted to areas of the United States where freezing winter temperatures would be lethal to *N. conspurcatalis*. It was concluded that *N. conspurcatalis* would pose no threat to native or cultivated plants in North America or the Caribbean and should be considered for biocontrol of *L. microphyllum*. A release petition was submitted in 2005. An USDA-APHIS release permit for *N. conspurcatalis* was issued in 2007.

Keywords: *Neomusotima conspurcatalis*; host range; *Lygodium microphyllum*

Introduction

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae), is native to tropical regions of Africa, Asia, Australia and the Pacific Islands (Pemberton 1998). *Lygodium microphyllum* is a rapidly growing, vine-like fern. The aerial parts of the plant are actually specialized climbing leaves that originate from the dorsal surface of a subterranean rhizome, which is actually the true stem (Mueller 1982). A rapidly growing leaf apex capable of indeterminate growth gives

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rise to a sclerified 'stem-like' rachis up to 30 m long that bears lateral leaves, called pinnae at regular intervals (Mueller 1983). Each pinna consists of a short stalk or petiolule, bearing two opposite, pinnate-pinnatifid pinnules and a dormant terminal leafbud (Mehlreter 2006).

Lygodium microphyllum was first found naturalized in Florida in the vicinity of the City of Jupiter, in Martin County, in 1968 (Beckner 1968), but since that time has spread rapidly to infest moist habitats throughout southern and central Florida. The fern readily climbs over trees and shrubs, smothering and excluding native understorey vegetation (Nauman and Austin 1978; Pemberton and Ferriter 1998) and changing local fire ecology (Pemberton and Ferriter 1998). Recent estimates of the area of land infested by *L. microphyllum* in southern Florida exceed 48,000 ha (Ferriter and Pernas 2006) but it is thought that the actual area infested is likely to be significantly higher (Volin, Lott, Muss, and Owen 2004). Most natural areas in south-central Florida are believed to be vulnerable to invasion by *L. microphyllum* (Goolsby 2004; Volin et al. 2004) and consequently this weed poses a substantial threat to Florida's native plants and ecosystems (Pemberton and Ferriter 1998).

Managing *L. microphyllum* has proved to be extremely difficult and costly (Hutchinson, Ferriter, Serbesoff-King, Langeland, and Rodgers 2006). Mechanical removal or burning are not viable strategies for long-term management because the fern readily regrows from rhizomes (Stocker, Ferriter, Thayer, Rock, and Smith 1997; Goolsby, Wright, and Pemberton 2003). Herbicides provide effective short-term control of *L. microphyllum*, but costly follow-up treatments are required (Hutchinson et al. 2006). High treatment costs (Pemberton, Goolsby, and Wright 2002; Thomas and Brandt 2003; Hutchinson et al. 2006) coupled with the remoteness and sheer extent of present *L. microphyllum* infestations make reliance on herbicides for long-term management unfeasible. Development of effective biological control options for *L. microphyllum* is vital to long-term, sustainable management of this weed in Florida (Pemberton 1998; Boughton and Pemberton 2008).

Scientists of the United States Department of Agriculture, Agricultural Research Service and Commonwealth Scientific and Industrial Research Organization (CSIRO) have collaboratively undertaken foreign exploration within the native range of *L. microphyllum* to identify suitable natural enemies for use in biological control programs (Goolsby et al. 2003). *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae) is a member of the fern-feeding subfamily Musotiminae (Solis, Yen, and Goolsby 2004). *Neomusotima conspurcatalis* was first discovered feeding on *L. microphyllum* in Hong Kong in 1997 (Goolsby et al. 2003). Subsequent foreign exploration for potential biocontrol agents of *L. microphyllum* revealed populations of *N. conspurcatalis* feeding on the target plant in Thailand, Malaysia, Singapore and down through Indonesia into the states of Western Australia and the Northern Territory. Specimens in The British Museum of Natural History also indicate that multiple collections of this insect were made in the state of Assam in northeast India during the late nineteenth century (Solis et al. 2004). Molecular diagnostic methods showed no variation in D2 expansion domain sequences of 28S rRNA from *N. conspurcatalis* specimens collected throughout this geographic range (Goolsby et al. 2003). *Neomusotima conspurcatalis* occurs from 15 degrees south in northern parts of Western Australia and the Northern Territory, to as far as 27 degrees north in northeastern India. The climate in northern regions of Australia is tropical, with average annual minimum temperatures from 5.0 to 10.0°C, corresponding

approximately to USDA cold hardiness zone 11 (ANBG 1991). At the northern end of the range of *N. conspurcatalis* in Assam in northeastern India, the climate is subtropical, with average annual minimum temperatures of -1.1 to 4.4°C , corresponding to USDA cold hardiness zone 10 (Widrlechner 1997). Between these northern and southern limits of the geographic range of *N. conspurcatalis*, climates are more tropical, with average monthly temperatures in summer of 30°C or higher and few if any days during the year with temperatures below 0°C . Bioclimatic modeling using the climate software CLIMEX confirmed the general similarity of conditions in these areas in Southeast Asia and northern Australia to the climate of southern Florida (Goolsby 2004).

Female *N. conspurcatalis* moths lay eggs on the upper or lower surface of *L. microphyllum* pinnules. After hatch, young larvae feed by scraping away cells on the undersurface of the pinnules until only a thin transparent layer of cells remain, giving rise to distinctive 'clear windows' in the pinnules. These transparent, scraped areas subsequently turn brown and the pinnules die. Older larvae consume whole pinnules. At high larval densities pronounced pinnule damage and defoliation can occur on lygodium plants. Larvae pupate on the above ground parts of the plant. Adults are small, short-lived, brown moths with a distinctive white 'boomerang-shaped' marking at the tip of each forewing. Female moths mate on the first night after emergence and typically commence oviposition on the next night. At 24°C , the life cycle from egg to adult typically takes about 30 days, and it is anticipated that this will translate into 10–12 field generations per year in Florida. *Neomusotima conspurcatalis* is multi-voltine, rearing continuously year-round under varying day lengths and natural light regimes, with no evidence of a diapausing, or cold-tolerant phase.

Although other *Lygodium* species including Japanese climbing fern, *L. japonicum* (Thunb.) Sw., *L. flexuosum* (L.) Sw., *L. reticulatum* Schkuhr and *L. circinnatum* (Burm.) are sympatric with *L. microphyllum* in certain parts of its native range, neither *N. conspurcatalis* larvae, nor the characteristic 'pinnule window' feeding damage they cause were ever observed on these other *Lygodium* species in the field (Goolsby et al. 2003). Limited host range testing had previously been conducted in Australia (Goolsby et al. 2003) on *N. conspurcatalis* and two other closely related moths in the subfamily Musotiminae, *Cataclysta camptozonale* (now *Austromusotima camptozonale* (Hampson) (Yen, Solis, and Goolsby 2004)) and *Musotima* sp. (now *Lygomusotima stria* Solis & Yen (Solis et al. 2004)). Plants tested in these no-choice development studies were selected to provide broad representation of fern families occurring in the tropics. *Neomusotima conspurcatalis* was tested on 12 species of fern, but development to adult only occurred on three *Lygodium* species, *L. microphyllum*, *L. japonicum* and American climbing fern, *Lygodium palmatum* (Bernhardi) Swartz, with high levels of survival occurring only on *L. microphyllum*. *Austromusotima camptozonale* was tested on 28 species of fern, but full development only occurred on four *Lygodium* species, *L. microphyllum*, *L. japonicum*, *L. palmatum* and *L. reticulatum*. Meanwhile, *L. stria* was tested on 12 species of fern, but full development only occurred on three *Lygodium* species, *L. microphyllum*, *L. japonicum* and *L. palmatum*. Based on these results, it was concluded that *N. conspurcatalis* was likely a fern specialist and in all probability restricted to the genus *Lygodium* and as such should be evaluated as a potential biological control agent of *L. microphyllum*.

Described herein are host range studies that were conducted with *N. conspurcatalis* in quarantine in Florida to determine on which plant species oviposition, feeding and development would occur. Simple low temperature survival studies were conducted to measure the susceptibility of *N. conspurcatalis* to brief exposure to sub-freezing temperatures, as would likely be encountered in occasional overnight frosts during the winter season in south-central Florida. Results of the laboratory host range studies and low temperature survival data for *N. conspurcatalis* are discussed with reference to plant hardiness zones of average annual minimum temperatures (Cathey 1990). It was concluded that *N. conspurcatalis* had an appropriate host specificity profile to warrant its consideration as a biocontrol agent for classical biological control of *L. microphyllum*.

Materials and methods

Neomusotima conspurcatalis populations tested

Initial host range screening studies conducted by USDA-ARS and CSIRO scientists at the USDA-ARS Australian Biological Control Laboratory (ABCL), CSIRO Long Pocket Laboratories, Indooroopilly, Queensland, Australia, used *N. conspurcatalis* from a population collected at Wangi Falls in Litchfield National Park in the Northern Territory of Australia (13°09'S, 130°38'E) during April 1999 (ABCL # 1999228). Fifty-five late instar larvae from this Wangi Falls collection were shipped to the United States in August 2001 and used to establish the initial quarantine colony at the Florida Biological Control Quarantine laboratory in Gainesville, Florida. However, this initial colony became infected with a microsporidian pathogen in June 2002 and this colony was subsequently terminated. A new *N. conspurcatalis* colony was established in October 2002 using 290 pupae derived from a field collection that was made at Thompson Springs near Kununurra, Western Australia (15°46'S, 128°44'E) during September 2002 (ABCL # 2002277). The vast majority of the comprehensive host range testing reported herein was conducted on this second *N. conspurcatalis* colony.

Plant species tested and rationale

A decision was made to proceed with full host range testing of *N. conspurcatalis* in quarantine in Florida. Thirty-seven species of non-*Lygodium* ferns broadly representative of the different families of ferns occurring in southern Florida, encompassing both Florida native species and species from other geographic regions commonly grown as horticultural plants, were selected as test plants. A special effort was also made to include in testing, species of ferns considered by The State of Florida to be threatened (*Athyrium filix-femina*, *Tectaria heracleifolia*, *Nephrolepis biserrata*, and *Pteris bahamensis*) or endangered (*Anemia wrightii*, *Ctenitis sloanei*, *Tectaria fimbriata* and *Actinostachys pennula*) (Appendix 1). Six *Lygodium* species in addition to *L. microphyllum* were included in testing. Two of these species, *L. japonicum* and *L. palmatum* occur in the United States. *Lygodium japonicum* is native to Asia, but has become an invasive weed in the United States (Van Loan 2006). Within its native range, *L. japonicum* occurs from India and Nepal eastward through Southeast Asia into Australia and ranges through China as far north as

Japan and Korea. Within the United States, *L. japonicum* is naturalized to the warmer coastal regions of all states from Virginia to Texas and is now common in northern and central Florida. *Lygodium japonicum* is also naturalized in Hawaii and Puerto Rico. *Lygodium palmatum* is the only *Lygodium* species native to the United States and has a temperate distribution throughout the eastern United States from Michigan in the north, down through the Appalachian states of Kentucky and Tennessee, with the southern limit of its current range corresponding to the northern counties of South Carolina, Georgia and Alabama, at a latitude of approximately 34.5 degrees north (Pemberton 1998; Pemberton et al. 2002). The other four *Lygodium* species, *L. cubense* Kunth, *L. oligostachyum* (Willdenow) Des, *L. venustum* Sw. and *L. volubile* Sw. occur in the Caribbean, Central and South America and are potential non-targets of *N. conspurcatalis*. Three plant species with importance to Florida agriculture, rice, citrus (orange) and sugarcane, were tested, as was bald cypress, an ecologically dominant, gymnosperm tree species common in south Florida habitats invaded by *L. microphyllum*. In total, 47 species of test plant, plus the primary host, *Lygodium microphyllum*, were evaluated in quarantine experiments for a total of 48 plant species (Appendix 1).

Source of plants

Lygodium microphyllum plants were dug from sites near the City of Jupiter or grown from spore collections made at the same sites. Related *Lygodium* species were field collected from various locations including: *Lygodium palmatum* from near Knoxville, TN; *L. volubile* from Argentina; and *L. venustum* and *L. oligostachyum* from the Dominican Republic. Plants of *L. cubense* were grown from spores from Cuba. Most of the other species of test plants were field collected in southern Florida or purchased from local nurseries or fern growers. Some species were obtained from the area around Gainesville, FL either by field collection or by purchase from local suppliers.

Colony maintenance

Neomusotima conspurcatalis eggs, larvae and pupae were reared in the quarantine laboratory on the bench top at 21.6°C, 45–85% relative humidity, and a photoperiod of 9 h L:15 h D. Pupae received from ABCL were placed on paper towels in a wooden, glass-topped sleeve cage 50 × 46 × 53 cm (l × w × h) in the quarantine laboratory until moths emerged. Adult moths were collected using a handheld, battery-powered, aspirator (Hausherr's Machine Works, Toms River, NJ) and were transferred for mating into small, collapsible sleeve cages 30 × 30 × 30 cm (l × w × h) (BioQuip, Rancho Dominguez, CA) in the quarantine greenhouse at 22.6–32.0°C, under natural light, supplemented with fluorescent grow-lights (Supersaver Daylight Deluxe FS-40X/ss) at 16 h L:8 h D. Each of these mating cages contained 10–20 adults and an oviposition sprig, consisting of a bouquet of *L. microphyllum* foliage with the cut rachis stems wrapped in cotton wool and inserted into a 185-mL plastic vial (Thornton Plastics, Salt Lake City, UT) of water, and a food source, consisting of a 26-mL glass vial containing a 9:1 Gatorade® [Lemon-Lime]: honey mixture, topped with a cotton dental wick (Carolina Cotton, Gaffney, SC). Gatorade® contains sucrose, glucose and fructose. These sugars and honey have frequently been used to supplement diets of adult female Lepidoptera in captivity to act as a source

of sugars, amino acids and vitamins they might otherwise have obtained from extra floral nectaries or homopteran honeydew in the environment (Tisdale and Sappington 2001; Romeis and Wackers 2002). Moths were allowed to mate inside the cages and oviposit on oviposition bouquets. Every few days, oviposition sprigs were removed from mating cages and replaced with fresh sprigs. After removal from mating cages, foliage from oviposition sprigs was transferred back into the quarantine lab into plastic, food-storage boxes $23 \times 17 \times 6$ cm containing fresh *L. microphyllum* foliage that had been collected weekly from field sites in Palm Beach and Martin counties in southern Florida. Following collection, plant material was soaked for 10 min in 10% (v/v) Clorox® bleach in water, and subsequently rinsed five times in clean water. Washed foliage was drained and packaged in clean 18-L plastic bags, and stored at 4°C until needed. Larvae emerging from eggs were allowed to develop, pupate and subsequently emerge as adults within the rearing boxes. Emerging adult moths were collected in a bench top light box, using a handheld, battery-powered, aspirator and were transferred to cages in the quarantine greenhouse and used either to maintain the colony or were used in the host testing experiments described below. When necessary for experiments, adults were sexed visually based on external genitalic characters (Solis et al. 2004).

Multi-choice oviposition tests on foliage bouquets

Multi-choice oviposition experiments using separate controls (choice-minus design (Schaffner 2001)), were utilized to rapidly screen a large number of test plants, to identify potential host species for subsequent testing in no-choice larval feeding and development studies. Experiments were conducted in wooden, glass-topped sleeve cages ($50 \times 46 \times 53$ cm or $74 \times 46 \times 53$ cm, $l \times w \times h$) in a quarantine greenhouse at 22.6–32.0°C, under natural light, supplemented with fluorescent grow-lights at 16 h L:8 h D, as previously described. Test cages contained from 4 to 12 bouquets of cut plant foliage, each bouquet consisting of cut foliage from a single species of test plant. A wide diversity of plant morphologies were represented among the test species, from small aquatic ferns to large free-standing terrestrial ferns, but to the extent possible, efforts were made to standardize the amount of foliage presented, by making bouquets approximately ‘fist-shaped’ in size. Experiments used a paired control consisting of a cage of the same size, positioned adjacent to the test cage, containing an equivalent number of bouquets of fresh cut *L. microphyllum* foliage. Foliage bouquets of test plants and *L. microphyllum* were made up in vials as described above. Bouquets were arranged in two parallel rows, along the length of the cage, with half the bouquets in each row, so that each row was equidistant from the side of the cage and the adjacent row. All cages were infested with 10 newly emerged male and 10 newly emerged female moths and contained a Gatorade®: honey food source as described above. Experiments were multi-choice, and allowed female *N. conspurcatalis* moths to demonstrate their oviposition preference, by choosing between bouquets of different species of test plant. Following setup, cages were covered in shade cloth to reduce entry of sunlight into the cages. Experiments were left running until all 10 females in the cage had died. Oviposition bouquets were examined and any eggs counted, and were then held in the laboratory to check for egg hatch and larval feeding. Experiments were replicated three times. Forty-six plant species were evaluated in

these multi-choice oviposition tests. *Actinostachys pennula* was not available at the time when these experiments were performed.

Multi-choice larval feeding and survival tests on cut foliage

Multi-choice larval feeding experiments using separate controls (choice-minus design (Schaffner 2001)) were utilized to rapidly screen a large number of test plants, to identify potential host species for subsequent testing in no-choice larval feeding and development studies. Studies were performed on the bench top in a quarantine lab at 21.6°C under 9 h L:15 h D. Experiments were conducted in clear plastic boxes (8 × 8 × 5 cm, 1 × w × h), the bottoms of which were lined with moistened paper towels. Each test box contained five portions of foliage, each approximately 900 mm² in size, from five species of test plant. Paired controls used identical boxes but contained five 900-mm² portions of *L. microphyllum* foliage. Three fourth instar *N. conspurcatalis* larvae were placed in each box, and feeding damage was assessed after 3 days. For test boxes, the area of leaf feeding damage on each plant species was estimated using a mm² grid. Due to heavy feeding on *L. microphyllum*, the area of foliage consumed in control boxes was calculated by multiplying the total number of leaves eaten by the area of a typical *L. microphyllum* leaf. For each control box, this total area was divided by 5 to yield an equivalent area of consumption per 900-mm² portion. Tests and paired controls were replicated 15 times. Thirty-seven plant species were evaluated in these multi-choice larval feeding tests. Five plant species (*Platycerium bifurcatum*, *Selaginella uncinata*, *Nephrolepis cordifolia*, *Phlebodium aureum*, *Anemia wrightii*) were excluded because they were not oviposition hosts. Five other species (*Actinostachys pennula*, *L. japonicum*, *Polystichum acrostichoides*, *Pteris vittata*, and *Ophioglossum petiolatum*) were omitted because they were not available at the time experiments were conducted.

No-choice development tests on potted plants

Experiments were conducted in aluminum-framed, screen cages (31 × 31 × 61 cm, d × w × h) in a quarantine greenhouse at 22.6–32.0°C, under natural light, supplemented with fluorescent grow-lights at 16 h L:8 h D, as previously described. Test cages contained a single, potted test plant. Control cages were positioned adjacent to test cages and contained a single potted *L. microphyllum* plant. The only exception to this were tests with *A. pennula*, in which tests and controls were housed in wooden, glass-topped sleeve cages (50 × 46 × 53 cm or 74 × 46 × 53 cm, l × w × h), owing to the large size of the *Osmunda cinnamomea* root masses on which the small *A. pennula* plants were growing. One replicate of the *A. pennula* tests used bouquets of cut *L. microphyllum* foliage inside control cages instead of potted plants. Test cages and paired control cages were inoculated with equivalent numbers of mating pairs of newly emerged *N. conspurcatalis* adults. Cages contained a Gatorade®: honey food source for moths as described above. Initially cages were infested with 10 mating pairs, but subsequently cages were infested with five mating pairs in order to reduce feeding damage on difficult to obtain test plants. Following setup, cages were covered in shade cloth to reduce entry of sunlight into the cages and moths were allowed to mate and oviposit on plants. Larvae emerging from the resulting eggs were allowed to feed and subsequently pupate on plants in the test and control cages. Numbers of adults that

subsequently emerged in test and control cages were recorded. Experiments were replicated from one to three times. On account of low levels of oviposition and larval feeding observed in previous multi-choice tests on non-*Lygodium* species, no-choice development tests were restricted to *Lygodium* species. However, *A. pennula* was included in these tests because it had not been available during earlier rounds of testing.

Low temperature survival tests

Simple studies were conducted to investigate the tolerance of *N. conspurcatalis* to short exposures to low and freezing temperatures as might be experienced in Florida in January and February during short, overnight frost events. Low temperature survival studies were conducted using a Frigomix B[®] refrigerated bath (Sartorius Biotech, Aubagne, France) filled with antifreeze solution. *Neomusotima conspurcatalis* pupae were placed individually in 2-mL glass vials with plastic screw cap lids, and totally immersed in the refrigeration bath for a period of 2 h. Thirty pupae were exposed at each of nine temperatures from 7 to -7°C. A control treatment consisted of 30 pupae in sealed vials, placed on the benchtop next to the Frigomix refrigerated bath at ambient lab temperature of 25°C for 2 h. Following exposure, pupae were incubated at 25°C until adults emerged. Percentage survival was calculated for pupae exposed at the nine temperatures and in the control treatment.

Statistical methods

Data were analyzed with SPSS statistical software (SPSS Inc., Chicago, IL). General Linear Models for the analysis of variance (ANOVA) were used to investigate the effect of plant species on variation in oviposition and feeding damage. Replicate and plant species were included in models as main effects, although replicate was included primarily as a blocking factor to reduce unexplained error variance. Dunnett's *post-hoc* test was used to evaluate differences between control and experimental means. Data were checked for conformity to ANOVA's underlying assumptions of normality of error and homogeneity of variance by examining plots of residuals and predicted values. Data from the larval feeding studies were square-root transformed prior to analysis to fix problems of heterogeneity of variance, but the means test results were unchanged relative to untransformed data, so means and standard errors for the untransformed data are presented in Table 2. In feeding trials, differences in mean rates of larval survival between control and experimental treatments were evaluated using independent sample *t*-tests. Results of the temperature studies were plotted graphically, and a trend line fitted using the 'moving average' procedure of Microsoft Excel (Microsoft Corporation[®], Redmond, WA). Linear regression was used to analyze the effect of temperature on moth survival over that portion of the temperature range where the relationship was linear.

Results

Multi-choice oviposition tests

During oviposition trials, female *N. conspurcatalis* laid the vast majority of eggs on plant material in the foliage bouquets. What little 'non-plant' oviposition that

Table 1. Multi-choice oviposition tests on foliage bouquets.

Plant species ¹	Experiment ²	No. eggs laid/bouquet ³	
		Test plants Mean ± SE	<i>L. microphyllum</i> control ⁴ Mean ± SE
<i>Lygodium japonicum</i>	A	372.7 ± 63.9 b	}
<i>Lygodium volubile</i>	A	130.7 ± 13.4 a	}
<i>Lygodium oligostachyum</i>	A	110.7 ± 59.2 a	} 176.3 ± 28.0 a
<i>Lygodium venustum</i>	A	81.0 ± 45.5 a	}
<i>Lygodium palmatum</i>	A	22.7 ± 14.0 c	}
<i>Lygodium cubense</i>	A	15.7 ± 15.2 c	}
<i>Osmunda cinnamomea</i>	B	7.3 ± 5.9 b	}
<i>Blechnum serrulatum</i>	B	4.3 ± 4.3 b	}
<i>Adiantum capillus-veneris</i>	B	1.3 ± 1.3 b	}
<i>Woodwardia virginica</i>	B	0.7 ± 0.7 b	}
<i>Anemia adiantifolia</i>	B	0.0 ± 0.0 b	}
<i>Asplenium platyneuron</i>	B	0.0 ± 0.0 b	} 44.3 ± 16.5 a
<i>Athyrium filix-femina</i>	B	0.0 ± 0.0 b	}
<i>Ctenitis sloanei</i>	B	0.0 ± 0.0 b	}
<i>Cyathea cooperi</i>	B	0.0 ± 0.0 b	}
<i>Nephrolepis biserrata</i>	B	0.0 ± 0.0 b	}
<i>Pteris bahamensis</i>	B	0.0 ± 0.0 b	}
<i>Thelypteris kunthii</i>	B	0.0 ± 0.0 b	}
<i>Osmunda regalis</i>	C	24.3 ± 23.8 b	}
<i>Asplenium nidus</i>	C	0.0 ± 0.0 b	}
<i>Marsilea vestita</i>	C	0.0 ± 0.0 b	}
<i>Pleopeltis polypodioides</i>	C	0.0 ± 0.0 b	} 55.6 ± 12.3 a
<i>Rumohra adiantiformis</i>	C	0.0 ± 0.0 b	}
<i>Selaginella pallescens</i>	C	0.0 ± 0.0 b	}
<i>Tectaria fimbriata</i>	C	0.0 ± 0.0 b	}
<i>Tectaria heracleifolia</i>	C	0.0 ± 0.0 b	}
<i>Woodwardia areolata</i>	C	0.0 ± 0.0 b	}
<i>Dryopteris ludoviciana</i>	D	102.0 ± 95.6 a	}
<i>Osmunda regalis</i>	D	25.7 ± 25.7 b	}
<i>Pteris vittata</i>	D	7.3 ± 5.0 b	}
<i>Cyrtomium falcatum</i>	D	6.3 ± 4.1 b	}
<i>Polystichum acrostichoides</i>	D	5.7 ± 5.7 b	}
<i>Pteridium aquilinum</i>	D	4.3 ± 2.2 b	} 82.3 ± 2.0 a
<i>Nephrolepis biserrata</i>	D	3.3 ± 3.3 b	}
<i>Anemia wrightii</i>	D	0.0 ± 0.0 b	}
<i>Asplenium scolopendrium</i>	D	0.0 ± 0.0 b	}
<i>Equisetum hyemale</i>	D	0.0 ± 0.0 b	}
<i>Platyserium bifurcatum</i>	D	0.0 ± 0.0 b	}
<i>Selaginella uncinata</i>	D	0.0 ± 0.0 b	}
<i>Ophioglossum petiolatum</i>	E	4.3 ± 4.3 b	}
<i>Saccharum officinarum</i>	E	1.0 ± 1.0 b	} 176.8 ± 41.5 a

Table 1. (Continued).

Plant species ¹	Experiment ²	No. eggs laid/bouquet ³	
		Test plants Mean ± SE	<i>L. microphyllum</i> control ⁴ Mean ± SE
<i>Citrus sinensis</i>	E	0.0 ± 0.0 b	}
<i>Oryza sativa</i>	E	0.0 ± 0.0 b	}
<i>Dryopteris ludoviciana</i>	F	9.7 ± 9.7 b	}
<i>Azolla caroliniana</i>	F	0.0 ± 0.0 b	}
<i>Nephrolepis cordifolia</i>	F	0.0 ± 0.0 b	} 62.5 ± 5.8 a
<i>Phlebodium aureum</i>	F	0.0 ± 0.0 b	}
<i>Salvinia minima</i>	F	0.0 ± 0.0 b	}
<i>Taxodium distichum</i>	F	0.0 ± 0.0 b	}

¹Species in bold type are native to Florida.
²Species with the same letter were tested in the same experiment, in the same cage. Each experiment had a test and control cage. Cages were infested with 10 male and 10 female moths. Experiments were replicated three times.
³Means based on three replicates.
⁴To achieve equivalent oviposition pressure, each control cage contained the same number of *L. microphyllum* bouquets as there were test bouquets in the test cage. For each replicate, average number of eggs per *L. microphyllum* bouquet was calculated by dividing total number of eggs on all *L. microphyllum* bouquets by the number of bouquets in the cage. Means and standard errors for controls were calculated across the three replicate averages using $n = 3$.

occurred was distributed equally between the bouquet vials and the sides of the cages. *Neomusotima conspurcatalis* females did not oviposit to a significant degree on non-*Lygodium* test species (Table 1). No oviposition occurred on cypress, rice, citrus or 69% (25 of 36) of the non-*Lygodium* fern species evaluated. When eggs were laid on non-*Lygodium* species, mean oviposition totals were low and were significantly less than those observed on *L. microphyllum*. In one set of experiments with *Dryopteris ludoviciana* (Table 1, Exp. D), the mean egg total exceeded that on the associated *L. microphyllum* control, but this was due to an extreme, outlying data point in the second replicate of the experiment, in which 293 eggs were laid on *D. ludoviciana*. This outlier was more than 17 standard deviations greater than the mean oviposition total obtained in a second experiment with *D. ludoviciana* (Table 1, Exp. F), where mean oviposition calculated from three replicates (9.7 ± 9.7 eggs) was only 16% of that observed on the *L. microphyllum* control. Overall, among six replicates with *D. ludoviciana*, no eggs were laid in three replicates, in two other replicates, 13 and 29 eggs were laid, respectively, and only in the remaining replicate was the high number of 293 eggs laid. Oviposition occurred on all six *Lygodium* test species and although egg totals were higher than those seen on non-*Lygodium* species, mean egg totals were generally 10–75% of those observed on *L. microphyllum*. Mean oviposition totals on *L. palmatum* and *L. cubense* were significantly lower than on *L. microphyllum*. Oviposition totals on *L. volubile*, *L. oligostachyum* and *L. venustum* were also lower than on *L. microphyllum*, although not by a statistically significant degree. The notable exception was *L. japonicum*, which proved to be preferred as an oviposition host over *L. microphyllum*. Mean oviposition on *L. japonicum* was significantly higher than on *L. microphyllum*, with about twice as many eggs being laid.

Table 2. Multi-choice larval feeding and survival tests on cut foliage.

Plant species ¹	Experiment ²	Leaf area consumed per 900 mm ² portion/(mm ²) ³		No. surviving larvae ⁴	
		Test plants Mean ± SE	<i>L. microphyllum</i> control Mean ± SE	Test plants Mean ± SE	<i>L. microphyllum</i> control Mean ± SE
<i>Lygodium volubile</i>	G	58.8 ± 9.1 b	}	}	}
<i>Lygodium palmatum</i>	G	35.0 ± 11.1 b	}	}	}
<i>Lygodium cubense</i>	G	20.6 ± 6.9 b	}	255.7 ± 16.6 a	1.3 ± 0.3 A
<i>Lygodium oligostachyum</i>	G	20.5 ± 4.1 b	}	}	2.9 ± 0.1 B
<i>Lygodium venustum</i>	G	11.7 ± 6.5 b	}	}	}
<i>Woodwardia virginica</i>	H	7.8 ± 2.1 b	}	}	}
<i>Osmunda cinnamomea</i>	H	5.1 ± 2.2 b	}	}	}
<i>Osmunda regalis</i>	H	2.1 ± 0.8 b	}	205.6 ± 18.0 a	2.1 ± 0.3 A
<i>Blechnum serrulatum</i>	H	0.0 ± 0.0 b	}	}	2.9 ± 0.1 B
<i>Rumohra adiantiformis</i>	H	0.0 ± 0.0 b	}	}	}
<i>Adiantum capillus-veneris</i>	I	13.5 ± 3.4 b	}	}	}
<i>Tectaria heracleifolia</i>	I	7.5 ± 2.5 b	}	}	}
<i>Woodwardia areolata</i>	I	5.2 ± 1.0 b	}	344.8 ± 33.7 a	1.7 ± 0.2 A
<i>Nephrolepis biserrata</i>	I	0.4 ± 0.2 b	}	}	2.9 ± 0.1 B
<i>Selaginella pallescens</i>	I	0.0 ± 0.0 b	}	}	}
<i>Tectaria fimbriata</i>	J	2.9 ± 2.0 b	}	}	}
<i>Pteris bahamensis</i>	J	2.3 ± 0.8 b	}	}	}
<i>Thelypteris kunthii</i>	J	0.5 ± 0.3 b	}	241.2 ± 15.5 a	1.6 ± 0.3 A
<i>Dryopteris ludoviciana</i>	J	0.2 ± 0.1 b	}	}	2.9 ± 0.1 B
<i>Pleopeltis polypodioides</i>	J	0.0 ± 0.0 b	}	}	}
<i>Cyathea cooperi</i>	K	21.7 ± 4.2 b	}	}	}
<i>Marsilea vestita</i>	K	3.9 ± 3.9 b	}	}	}
<i>Anemia adiantifolia</i>	K	0.0 ± 0.0 b	}	179.7 ± 12.6 a	1.5 ± 0.3 A
<i>Athyrium filix-femina</i>	K	0.0 ± 0.0 b	}	}	2.7 ± 0.1 B
<i>Ctenitis sloanei</i>	K	0.0 ± 0.0 b	}	}	}
<i>Pteridium aquilinum</i>	L	3.3 ± 0.9 b	}	}	}
<i>Cyrtomium falcatum</i>	L	1.3 ± 0.6 b	}	}	}

Table 2. (Continued).

Plant species ¹	Experiment ²	Leaf area consumed per 900 mm ² portion/(mm ²) ³		No. surviving larvae ⁴	
		Test plants Mean \pm SE	<i>L. microphyllum</i> control Mean \pm SE	Test plants Mean \pm SE	<i>L. microphyllum</i> control Mean \pm SE
<i>Anemia adiantifolia</i>	L	0.1 \pm 0.1 b	} 169.6 \pm 20.5 a	} 1.6 \pm 0.3 A	} 2.8 \pm 0.1 B
<i>Asplenium nidus</i>	L	0.0 \pm 0.0 b	}	}	}
<i>Asplenium scolopendrium</i>	L	0.0 \pm 0.0 b	}	}	}
<i>Asplenium platyneuron</i>	M	9.9 \pm 4.3 b	}	}	}
<i>Salvinia minima</i>	M	2.5 \pm 1.1 b	}	}	}
<i>Taxodium distichum</i>	M	0.1 \pm 0.1 b	} 185.3 \pm 13.8 a	} 1.1 \pm 0.2 A	} 2.9 \pm 0.1 B
<i>Azolla caroliniana</i>	M	0.0 \pm 0.0 b	}	}	}
<i>Saccharum officinarum</i>	M	0.0 \pm 0.0 b	}	}	}
<i>Cyathea cooperi</i>	N	1.2 \pm 0.5 b	}	}	}
<i>Adiantum capillus-veneris</i>	N	0.9 \pm 0.5 b	}	}	}
<i>Citrus sinensis</i>	N	0.0 \pm 0.0 b	} 96.3 \pm 8.4 a	} 0.2 \pm 0.1 A	} 2.9 \pm 0.1 B
<i>Equisetum hyemale</i>	N	0.0 \pm 0.0 b	}	}	}
<i>Oryza sativa</i>	N	0.0 \pm 0.0 b	}	}	}

¹Plant species in bold type are native to Florida.

²Plant species with the same letter were tested in the same experiment, and were present in the same plastic box. Each experiment had a test box and paired control box. Experiments were replicated 15 times.

³Each test box contained approximately 900 mm² of each of the five test plant species. Each control box contained five 900-mm² portions of *L. microphyllum*. Boxes contained three fourth instar *N. conspurcatalis* larvae. Feeding damage was assessed after 3 days. For test plants, areas consumed were estimated using a mm² grid. Due to heavy feeding on controls, areas consumed were calculated by multiplying total number of leaves eaten by the area of a typical *L. microphyllum* leaf. For controls, total area was divided by 5 to yield an equivalent amount of consumption per 900 mm² portion. Means and standard errors based on data from 15 replicates. Within an experiment, test plant means are significantly different from the control mean by Dunnett's test ($P < 0.05$) if followed by different lower case letters.

⁴Number of larvae alive at the end of 3 days. Means and standard errors based on data from 15 replicates. Within an experiment, means followed by different upper case letters are significantly different by *t*-test ($P < 0.05$).

Multi-choice larval feeding and survival tests

Significant feeding by *N. conspurcatalis* larvae did not occur on non-*Lygodium* test plants (Table 2). No feeding occurred on rice, sugarcane, citrus or 39% (11 of 28) of non-*Lygodium* fern species. When feeding did occur on non-*Lygodium* species, the amounts of damage were extremely small and were always significantly less than on *L. microphyllum*. The observed feeding on test plants was consistent with 'trial nibbling' as larvae evaluated the palatability of foliage. Feeding did occur on all five *Lygodium* species that were evaluated, but the amounts of damage were low and in all cases were significantly less than on *L. microphyllum*. At the end of the 3-day feeding period, survival of *N. conspurcatalis* larvae was always significantly higher in *L. microphyllum* control boxes, typically averaging 97%, compared to survival rates ranging from 7–72% in test boxes containing foliage from other test plants.

No-choice development tests

In addition to on the primary host *L. microphyllum*, *N. conspurcatalis* was only capable of completing development to adult on *L. japonicum* and *L. palmatum* (Table 3). The number of *N. conspurcatalis* developing to adult on potted *L. japonicum* and *L. palmatum* plants were appreciably lower at only 54 and 40%, respectively, of the

Table 3. No-choice development tests on potted plants.

Species ¹	Experiment ²	Infestation pressure ³	No. <i>N. conspurcatalis</i> developing to adult/plant ⁴	
			Test plant Mean \pm SE	<i>L. microphyllum</i> control Mean \pm SE
<i>L. japonicum</i>	O	10 pairs	98.7 \pm 54.8 a	} 183.0 \pm 93.6 a
<i>L. palmatum</i>	O	10 pairs	74.0 \pm 15.7 a	}
<i>L. venustum</i>	P	5 pairs	0.0 \pm 0.0 b	} 169.0 \pm 70.6 a
<i>L. cubense</i>	P	5 pairs	0.0 \pm 0.0 b	}
<i>L. volubile</i>	Q	5 pairs	0.0 \pm 0.0 a	} 219.5 \pm 85.5 a
<i>L. oligostachyum</i>	R	5 pairs	0	} 134
<i>A. pennula</i> ⁵	S	10 pairs	0.0 \pm 0.0 a	} 49.7 \pm 21.7 a
<i>A. pennula</i>	T	30 eggs	0.0 \pm 0.0 a	} 18.3 \pm 9.1 a

¹Species in bold are Florida natives.

²Plant species with the same letter were tested in the same experiment. Experiments used one potted test plant per aluminum screen cage, and a paired control cage containing one potted *L. microphyllum* plant. Experiments replicated three times, except 'Q' which was replicated twice and 'R' which was performed only once.

³Test and control cages infested with 10 or five mating pairs of *N. conspurcatalis* adults. Female moths were allowed to oviposit on plants. In one experiment plants were infested with 30 *N. conspurcatalis* eggs.

⁴Within an experiment, test plant means are significantly different from the control mean ($P < 0.05$) by Dunnett's test (experiments 'O' and 'P') or t-test (experiments 'Q', 'S' and 'T') if followed by different lower case letters.

⁵Control cages contained three foliage bouquets of *L. microphyllum* rather than a potted plant.

number surviving to adult on potted *L. microphyllum* plants. There was no survival to adult of *N. conspurcatalis* on any of the other four *Lygodium* species tested, nor on *Actinostachys pennula*.

Low temperature survival tests

Regression analysis of low temperature survival data indicated a strong linear relationship (r -square 0.948) from 7 to -5°C , between declining temperatures and reductions in survival of *N. conspurcatalis* (Figure 1). The slope of the regression line was significantly different from zero ($t=9.53$; $\text{df}=5$; $P<0.01$) as was the intercept of the regression line ($t=53.6$; $\text{df}=5$; $P<0.01$). For the linear portion of the data range, from -5 to 7°C , the regression equation was 'survival = $77.24 + (3.15 \times \text{temperature})$ '. At temperatures below -5°C , the relationship between temperature and survival broke down, and survival declined steeply such that no *N. conspurcatalis* pupae exposed to temperatures below -7°C survived to the adult stage. At temperatures above 7°C , rates of survival were similar to those observed in control experiments at 25°C , and exceeded 86%.

Discussion

Results indicate that *N. conspurcatalis* is specific to the genus *Lygodium*. *Neomusotima conspurcatalis* did not oviposit or feed appreciably on the 37 species of non-*Lygodium* ferns representative of genera occurring in Florida, the Caribbean and elsewhere in the Americas. Several hundred eggs were laid on *D. ludoviciana* in one of the six replicates of the multi-choice oviposition tests, but since individual *N. conspurcatalis* females can lay up to 172 eggs (A.J. Boughton, unpublished

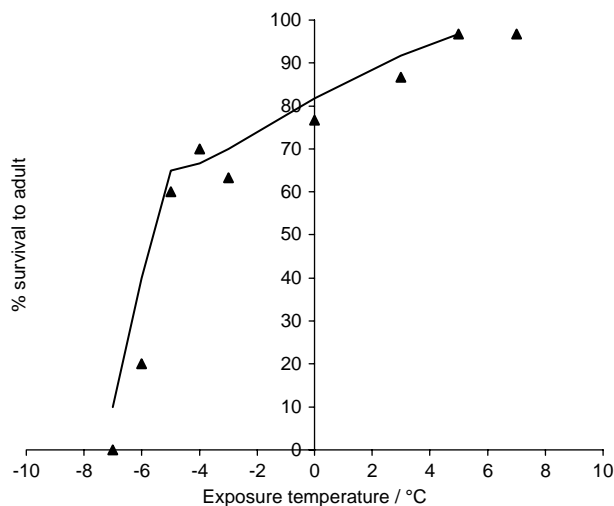


Figure 1. Affect of exposure to low temperatures on survival of *N. conspurcatalis*. Thirty *N. conspurcatalis* pupae were exposed for 2 h at each temperature, and subsequently maintained in the laboratory at 25°C until adult emergence. In controls, pupae maintained in vials at ambient laboratory temperature of 25°C exhibited 86.7% survival to adult. Trend line fitted using the 'moving average' procedure of Microsoft Excel.

data), this result on *D. ludoviciana* could have been caused by aberrant oviposition by as few as two female moths. Unusually high oviposition was also observed on *Osmunda regalis* in that replicate of the multi-choice oviposition studies. Fern species in the families Anemiaceae and Schizaeaceae are the closest relatives of ferns in the family Lygodiaceae (Pemberton et al. 2002; Wikstrom, Kenrick, and Vogel 2002), but even these close relatives were not utilized by *N. conspurcatalis*. No oviposition or larval feeding occurred on two *Anemia* spp. (Anemiaceae) and *N. conspurcatalis* was unable to complete development on *Actinostachys pennula* (Swartz) Hooker (Schizaeaceae). *Neomusotima conspurcatalis* did not oviposit or feed on bald cypress, which is a gymnosperm tree that commonly occurs as an ecologically dominant species in *Lygodium*-infested habitats in south Florida. Neither did oviposition or feeding by *N. conspurcatalis* occur on three important agricultural crops, rice, sugarcane and citrus (orange) that are widely grown in Florida.

Oviposition and feeding by *N. conspurcatalis* occurred on all six *Lygodium* test species, but complete development only occurred on *L. japonicum* and *L. palmatum*. However, it is difficult to reconcile these patterns of host use by *N. conspurcatalis* with species relationships within the genus *Lygodium*. Historically, relatedness between different *Lygodium* species has been inferred from taxonomies based on comparative plant morphology (Prantl 1881; Reed 1946). Prantl (1881) placed *L. microphyllum*, *L. reticulatum* and *L. volubile* in the subgenus Volubilia, the Asian species *L. flexuosum* and *L. japonicum* and Caribbean species *L. cubense* and *L. venustum* in the subgenus Flexuosa, and the American climbing fern *L. palmatum* in a small, basal subgenus Palmata, which was assumed to represent the ancestral state. Reed (1946) arrived at a similar classification, also recognizing three subgenera, although invoked a different ancestral form. This basic pattern of inter-relatedness between species within the genus *Lygodium* has been corroborated by recent molecular analyses using DNA sequence data from chloroplast genes (Wikstrom et al. 2002; Madeira, Pemberton, and Center 2008), except that *L. volubile* is placed in a large clade containing the Caribbean (*L. cubense*, *L. venustum*, *L. oligostachyum*) and Asian (*L. flexuosum*, *L. japonicum*, *L. circinnatum*) species. *Lygodium microphyllum* and another Asian species *L. reticulatum* are grouped in a sister clade of equal rank, while *L. palmatum* is again placed on its own as a basal offshoot that is only distantly related to the *L. microphyllum*/*L. reticulatum* and Caribbean/Asian species assemblages. Although it is relatively easy to explain host usage of *L. palmatum* and *L. microphyllum* by *N. conspurcatalis*, by assuming that suitability for *N. conspurcatalis* was an ancestral trait in the *Lygodium* lineage, it is difficult to explain why *N. conspurcatalis* develops on *L. japonicum*, but is unable to develop on closely related Caribbean or Asian *Lygodiums* that occur in the same subgenus. While not always predictive, it has frequently been noted that the ability of an insect herbivore to develop on a given plant species appears to be more likely when closely related herbivore species also use that same host plant (Ehrlich and Raven 1964; Futuyma 2000; Janz, Nyblom, and Nylin 2001). The existence of a congeneric moth species in East Asia, *Neomusotima fuscolinealis* Yoshiyasu, that is a specialist on *L. japonicum* (Bennett and Pemberton 2008), may partly explain why *N. conspurcatalis* is also able to utilize *L. japonicum*. Regardless of the reasons underlying acceptability, development of *N. conspurcatalis* on *L. japonicum* is not problematic in the context of host specificity of a potential biocontrol agent of *L. microphyllum*, because *L. japonicum* is itself an invasive weed in the United States. As such, feeding on *L. japonicum* by

N. conspurcatalis would not be viewed as a non-target problem. The exact pattern of host usage of *L. japonicum* by *N. conspurcatalis* is difficult to predict at this current time. Infestations of *L. japonicum* in Florida are distributed primarily in counties in the northern half of the Florida peninsula to the north and northwest of Lake Okeechobee (Van Loan 2006). Meanwhile the majority of the land area currently infested with *L. microphyllum* is located to the south and west of Lake Okeechobee. It is anticipated that populations of *N. conspurcatalis* will initially occur on *L. microphyllum* infestations in the southern half of the Florida peninsula, and so will not encounter significant infestations of *L. japonicum*. How *N. conspurcatalis* will behave in areas where both *L. microphyllum* and *L. japonicum* occur is not clear. Although lab tests indicated that *L. japonicum* is physiologically suitable as a development host, field surveys in Australia and Southeast Asia did not document *N. conspurcatalis* or its characteristic feeding damage on *L. japonicum*. So while *N. conspurcatalis* larvae may move onto neighboring *L. japonicum* plants when *L. microphyllum* plants have been defoliated, it remains to be seen whether *N. conspurcatalis* populations will develop in areas where only *L. japonicum* is present.

Low temperature survival studies conducted with *N. conspurcatalis* indicated 80% mortality of pupae following 2 h exposure at -6°C , while a similar duration of exposure at -7°C caused 100% mortality. Although basic, these studies predict that *N. conspurcatalis* would not be found inhabiting areas where winter temperatures were colder than USDA cold hardiness zone 9a (average annual minimum temperatures -6.6 to -3.9°C). These predictions are supported by what is known of the moth's actual distribution in its native range, which indicate that it is restricted to areas warmer than the Assam region of northeastern India, corresponding to USDA cold hardiness zone 10 (average annual minimum temperatures -1.1 to 4.4°C) (Widrechner 1997). The results of the temperature studies and what is known of the moths distribution in its native range suggest that in Florida, *N. conspurcatalis* is likely to be restricted to areas in USDA cold hardiness zone 9b (average annual minimum temperature -3.8 to -1.2°C) and warmer (USNA 2003). This broadly corresponds to the southern two-thirds of the Florida peninsula and areas to the south of a line from Daytona Beach (29°N) on the east coast, through Lake Placid (27.5°N) in the southern interior of the peninsula up to Tampa (28°N) on the west coast. This predicted distribution in Florida based on climatic data corresponds well with the known northerly limit of the distribution of *N. conspurcatalis* in its native range, where it occurs to the latitude of 27 degrees north in Assam in northeastern India (Solis et al. 2004). The northern part of the Florida peninsula (approximately 30°N) from Jacksonville on the Atlantic coast, west to Pensacola on the Gulf Coast, broadly corresponding to USDA Hardiness zone 8b (USNA 2003), where average annual minimum temperatures range from -9.4 to -6.7°C , would likely be lethal to *N. conspurcatalis* populations.

Lygodium palmatum was the only North American native plant that supported development of *N. conspurcatalis*. However, *Lygodium palmatum* is a temperate fern and significant populations are not known to exist further south than the northern counties of South Carolina, Georgia and Alabama, at a latitude of approximately 34.5 degrees north (Pemberton 1998; Pemberton et al. 2002). Three herbarium records for *L. palmatum* exist from slightly further south in South Carolina in coastal counties bordering North Carolina, but these occurrences were single, isolated plants and it is uncertain whether they reflect natural populations of the fern, since one was

a collection adjacent to a garden planting of *L. palmatum*, and the other two collections were associated with human disturbance, having been made from a drainage ditch and electric powerline right of way, respectively. Even these coastal counties in South Carolina are some 500 km further north than the northern-most limit of *N. conspurcatalis* in its native range and the climate in these coastal counties corresponds to cold hardiness zone 8a, where average annual minimum temperatures range from -12.2 to -9.5°C . The northern counties of South Carolina, Georgia and Alabama meanwhile are 100 km further north still, and the climate in this area corresponds to cold hardiness zone 7, where average annual minimum temperatures range from -17.8 to -12.2°C . Winter temperatures in any of these areas would cause 100% mortality of *N. conspurcatalis*. It was concluded that *N. conspurcatalis* would not be capable of inhabiting latitudes so far beyond the northern limit of its native range, where winters are substantially colder and longer than anywhere where it currently occurs.

In summary, *N. conspurcatalis* is predominantly a tropical moth that is specific to three fern species in the genus *Lygodium*. The moth will not harm native or cultivated plants. Although the moth does develop on the native climbing fern, *L. palmatum*, the moth and the fern have different climatic requirements, and their geographic ranges in North America are widely separated. If the moth somehow migrated to neighboring Caribbean islands, there would be no harm to the native *Lygodium* species that grow there, because they do not support development of the moth. Based on the comprehensive risk evaluation documented herein, we concluded that the host range of *N. conspurcatalis* was such that the moth would pose no threat to native or cultivated plants in the United States, and consequently that the moth should be considered as a potential biocontrol agent of the invasive, ecologically damaging weed, *L. microphyllum*. A release petition for *N. conspurcatalis*, containing summary data from the studies reported herein, was submitted to the Technical Advisory Group for Biological Control of weeds in 2005. The technical advisory group recommended release of *N. conspurcatalis* in 2006 and an USDA-APHIS release permit was issued in 2007.

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References

- ANBG (1991), 'Australian National Botanic Gardens Plant Hardiness Zones Map for Australia', <http://www.anbg.gov.au/hort.research/zones.html>.
- Beckner, J. (1968), '*Lygodium microphyllum*, another Fern Escaped in Florida', *American Fern Journal*, 58, 93–94.

- Bennett, C.A., and Pemberton, R.W. (2008), 'Neomusotima fuscolinealis (Lepidoptera: Pyralidae) is an Unsuitable Biological Control Agent of *Lygodium japonicum*', *Florida Entomologist*, 91, 26–29.
- Boughton, A.J., and Pemberton, R.W. (2008), 'Efforts to Establish a Foliage-feeding Moth, *Austromusotima camptozonale*, against *Lygodium microphyllum* in Florida, Considered in the Light of a Retrospective Review of Establishment Success of Weed Biocontrol Agents Belonging to Different Arthropod Taxa', *Biological Control*, 47, 28–36.
- Cathey, H.M. (1990), *USDA Plant Hardiness Zone Map (USDA Miscellaneous Publication No. 1475)*, Washington, DC: United States Department of Agriculture.
- Ehrlich, P.R., and Raven, P.H. (1964), 'Butterflies and Plants: A Study in Coevolution', *Evolution*, 18, 586–608.
- Ferriter, A., and Pernas, T. (2006), 'An Explosion in Slow Motion: Tracking the Spread of *Lygodium microphyllum* in Florida', *Wildland Weeds*, 9, 7–9.
- Flora of North America Editorial Committee (1993), *Flora of North America North of Mexico, Volume 2, Pteridophytes and Gymnosperms*. Oxford University Press, New York, pp. 475.
- Futuyma, D.J. (2000), 'Some Current Approaches to the Evolution of Plant-Herbivore Interactions', *Plant Species Biology*, 15, 1–9.
- Goolsby, J.A. (2004), 'Potential Distribution of the Invasive Old World Climbing Fern, *Lygodium microphyllum* in North and South America', *Natural Areas Journal*, 24, 351–353.
- Goolsby, J.A., Wright, A.D., and Pemberton, R.W. (2003), 'Exploratory Surveys in Australia and Asia for Natural Enemies of Old World Climbing Fern, *Lygodium microphyllum*: Lygodiaceae', *Biological Control*, 28, 33–46.
- Hoshizaki, B.J., and Moran, R.C. (2001), *Fern Grower's Manual*, Portland, OR: Timber Press.
- Hutchinson, J., Ferriter, A., Serbesoff-King, K., Langeland, K., and Rodgers, L. (2006). 'Old World Climbing Fern, *Lygodium microphyllum*, Management Plan for Florida' (available online <http://www.fleppc.org/>). Florida Exotic Pest Plant Council.
- Janz, N., Nyblom, K., and Nylin, S. (2001), 'Evolutionary Dynamics of Host Plant Specialization: A Case Study of the Tribe Nymphalini', *Evolution*, 55, 783–796.
- Madeira, P.T., Pemberton, R.W., and Center, T.D. (2008), 'A Molecular Phylogeny of the Genus *Lygodium* (Schizaeaceae) with Special Reference to the Biological Control and Host Range Testing of *Lygodium microphyllum*', *Biological Control*, 45, 308–318.
- Mehlreiter, K. (2006), 'Leaf Phenology of the Climbing Fern *Lygodium venustum* in a Semideciduous Lowland Forest on the Gulf of Mexico', *American Fern Journal*, 96, 21–30.
- Mueller, R.J. (1982), 'Shoot Morphology of the Climbing Fern *Lygodium* (Schizaeaceae): General Organography, Leaf Initiation, and Branching', *Botanical Gazette*, 143, 319–330.
- Mueller, R.J. (1983), 'Indeterminate Growth and Ramification of the Climbing Leaves of *Lygodium japonicum* (Schizaeaceae)', *American Journal of Botany*, 70, 682–690.
- Nauman, C.E., and Austin, D.F. (1978), 'Spread of *Lygodium microphyllum* in Florida', *American Fern Journal*, 68, 65–66.
- Pemberton, R.W. (1998), 'The Potential of Biological Control to Manage Old World Climbing Fern (*Lygodium microphyllum*), an Invasive Weed in Florida', *American Fern Journal*, 88, 176–182.
- Pemberton, R.W., and Ferriter, A.P. (1998), 'Old World Climbing Fern (*Lygodium microphyllum*), a Dangerous Invasive Weed in Florida', *American Fern Journal*, 88, 165–175.
- Pemberton, R.W., Goolsby, J.A., and Wright, T. (2002), 'Old World Climbing Fern', in *Biological Control of Invasive Plants in the Eastern United States (Publication FHTET-2002-04) – Forest Health Technology Enterprise Team*, eds R. Van Driesche, S. Lyon, B. Blossey, M. Hoddle and R. Reardon, USDA Forest Service, pp. 139–147.
- Prantl, K. (1881), 'Die Schizaeaceen', in *Morphologie der Gefasskryptogamen*, Vol. 2, Leipzig, Germany: Verlag Voon Wilhelm Engelmann, pp. 7–85.
- Reed, C.F. (1946), 'The Phylogeny and Ontogeny of the Pteropsida I. Schizaeales', *Boletim da Sociedade Broteriana*, 21, 71–197.
- Romeis, J., and Wackers, F.L. (2002), 'Nutritional Suitability of Individual Carbohydrates and Amino Acids for Adult *Pieris brassicae*', *Physiological Entomology*, 27, 148–156.

- Schaffner, U. (2001), 'Host Range Testing of Insects for Biological Weed Control: How Can it be Better Interpreted?', *BioScience*, 51, 951–959.
- Solis, M.A., Yen, S.-H., and Goolsby, J.A. (2004), 'Species of *Lygomusotima* New Genus and *Neomusotima* Yoshiyasu (Lepidoptera: Crambidae) from Australia and Southeastern Asia Feeding on *Lygodium microphyllum* (Schizaeaceae)', *Annals of the Entomological Society of America*, 97, 64–76.
- Stocker, R.K., Ferriter, A., Thayer, D., Rock, M., and Smith, S. (1997), 'L. *microphyllum* Hitting South Florida below the Belt', *Wildland Weeds*, 1, 6–10.
- Thomas, B., and Brandt, L.A. (2003), 'Monitoring Ground Treatments of Old World Climbing Fern (*Lygodium microphyllum*) on the Arthur R. Marshall Loxahatchee NWR', *Wildland Weeds*, 6, 9–11.
- Tisdale, R.A., and Sappington, T.A. (2001), 'Realized and Potential Fecundity, Egg Fertility, and Longevity of Laboratory-reared Female Beet Armyworm (Lepidoptera: Noctuidae) under Different Adult Diet Regimes', *Annals of the Entomological Society of America*, 94, 415–419.
- USNA (2003), The US National Arboretum Plant Hardiness zone map, USDA Miscellaneous Publication No. 1475: <http://www.usna.usda.gov/Hardzone/index.html>.
- Van Loan, A.N. (2006), 'Japanese Climbing Fern: The Insidious "Other" *Lygodium*', *Wildland Weeds*, 9, 25–27.
- Volin, J.C., Lott, M.S., Muss, J.D., and Owen, D. (2004), 'Predicting Rapid Invasion of the Florida Everglades by Old World Climbing Fern (*Lygodium microphyllum*)', *Diversity and Distributions*, 10, 439–446.
- Widrechner, M.P. (1997), 'Plant Hardiness Zones in China', <http://www.ars.usda.gov/Main/docs.htm?docid=9815&page=2>.
- Wikstrom, N., Kenrick, P., and Vogel, J.C. (2002), 'Schizaeaceae: A Phylogenetic Approach', *Review of Palaeobotany and Palynology*, 119, 35–50.
- Wunderlin, R.P., and Hansen, B.F. (2000), *Flora of Florida* 1, Pteridophytes and Gymnosperms, Gainesville, FL: University Press of Florida.
- Yen, S.-H., Solis, M.A., and Goolsby, J.A. (2004), '*Austromusotima*, a New Musotime Genus (Lepidoptera: Crambidae) Feeding on Old World Climbing Fern, *Lygodium microphyllum* (Schizaeaceae)', *Annals of the Entomological Society of America*, 97, 397–410.

Appendix 1. Test plant species.

FAMILY Species ¹	Common name	Native area ²	Reason tested ³
LYGODIACEAE			
<i>Lygodium microphyllum</i>	Old World climbing fern	Tropical Africa, SE Asia, Australia, E Indies	Target weed, Florida genotype
<i>Lygodium japonicum</i> (Thunb.) Sw.	Japanese climbing fern	E Asia, E USA	Congener, invasive weed
<i>Lygodium palmatum</i> (Bernhardi) Swartz	American climbing fern	E USA	Congener
<i>Lygodium cubense</i> Kunth	Cuban climbing fern	Cuba (endemic)	Congener
<i>Lygodium oligostachyum</i> (Willdenow) Des.	None	Hispaniola (endemic)	Congener
<i>Lygodium venustum</i> Sw.	None	W Indies to Brazil	Congener
<i>Lygodium volubile</i> Sw.	None	W Indies to Argentina	Congener
ANEMIACEAE			
<i>Anemia adiantifolia</i> (L.) Swartz	None Pine fern	S Florida, to Brazil	Related
<i>Anemia wrightii</i> Baker	Wright's pineland fern	S Florida, W Indies, C America	Florida endangered, related
ASPLENIACEAE			
<i>Asplenium platyneuron</i> (L.) Britton, Sterns & Poggenburg	Ebony spleenwort	Florida, USA, S Africa	Representative
<i>Asplenium scolopendrium</i> L.	Hart's-tongue fern	E USA, S Mexico, Hispaniola	Representative
<i>Asplenium nidus</i> L.	Bird's-nest fern	Tropical Africa, SE Asia,	Horticultural, representative
AZOLLACEAE			
<i>Azolla caroliniana</i> Willdenow	Mosquito fern	Florida, USA, C America, Europe, Asia	Representative
BLECHNACEAE			
<i>Blechnum serrulatum</i> Richard	Swamp water fern	Florida to S America	Horticultural, representative
<i>Woodwardia areolata</i> (L.) Moore	Netted chain fern	Florida, E USA	Representative
<i>Woodwardia virginica</i> (L.) Smith	Virginia chain fern	Florida, E USA	Representative
CYATHEACEAE			
<i>Cyathea cooperi</i> (Mueller) Domin	Australian tree fern	Australia	Representative
DENNSTAEDTIACEAE			
<i>Pteridium aquilinum</i> (L.) Kuhn	Bracken fern	Florida, worldwide	Representative

Appendix 1. (Continued).

FAMILY Species ¹	Common name	Native area ²	Reason tested ³
DRYOPTERIDACEAE			
<i>Athyrium filix-femina</i> Roth	Lady fern	Florida, circumboreal	Florida threatened, representative
<i>Ctenitis sloanei</i> (Poeppig ex Sprengel) Morton	Florida tree fern	Florida, W Indies, C & S America	Florida endangered, representative
<i>Cyrtomium falcatum</i> (L.f.) Presl	Japanese holly fern	E Asia, naturalized Florida	Horticultural, representative
<i>Dryopteris ludoviciana</i> (Kunze) Small	Southern wood fern	Florida, S USA	Representative
<i>Polystichum acrostichoides</i> (Michaux) Schott	Christmas fern	Florida, USA, Mexico	Representative
<i>Rumohra adiantiformis</i> (Forst) Ching	Leather leaf fern	Pantropical, New Zealand	Floral industry, representative
<i>Tectaria fimbriata</i> (Willdenow) Proctor & Lourteig	Least halberd	Florida, W Indies, Mexico	Florida endangered, representative
<i>Tectaria heracleifolia</i> (Willdenow) Underwood	Broad halberd	Florida, W Indies, C & S America	Florida threatened, representative
<i>Nephrolepis biserrata</i> (Swartz) Schott	Giant sword fern	Florida, W Indies, C & S America, Tropical Africa, SE Asia	Florida threatened
<i>Nephrolepis cordifolia</i> (L.) Presl	Tuberous sword fern	Pantropical, naturalized Florida	Weed, representative
EQUISETACEAE			
<i>Equisetum hyemale</i> L.	Rough horsetail	Florida, USA, Europe, Asia	Representative
MARSILEACEAE			
<i>Marsilea vestita</i> Hooker & Greville	Water clover	USA, Mexico, Peru	Representative
OPHIOGLOSSACEAE			
<i>Ophioglossum petiolatum</i> Hooker	Slender adders-tongue	Florida, W Indies, Mexico to S America, Asia	Representative
OSMUNDACEAE			
<i>Osmunda cinnamomea</i> L.	Cinnamon fern	Florida, USA, C & S America, Asia	Representative
<i>Osmunda regalis</i> L.	Royal fern	Florida, worldwide	Horticultural, representative
POLYPODIACEAE			
<i>Phlebodium aureum</i> (L.) Smith	Goldfoot fern	Florida, S USA to S America	Horticultural, representative
<i>Platynerium bifurcatum</i> (Cav.) C.Ch.	Staghorn fern	Australasia	Horticultural, representative

Appendix 1. (Continued).

FAMILY Species ¹	Common name	Native area ²	Reason tested ³
<i>Pleopeltis polypodioides</i> (L.) Andrews & Windham	Resurrection fern	Florida, E USA, W Indies, Mexico	Horticultural, representative
PTERIDACEAE			
<i>Adiantum capillus-veneris</i> L.	Southern maidenhair fern	Florida, worldwide	Horticultural, representative
<i>Pteris bahamensis</i> (Agardh) Fee	Bahama ladder brake	Florida, Bahamas	Florida threatened, representative
<i>Pteris vittata</i>	Chinese Ladder brake	Asia, naturalized Florida	Weed, representative
SALVINIACEAE			
<i>Salvinia minima</i> Baker	Water fern	Tropical America, naturalized Florida	Representative
SCHIZAEACEAE			
<i>Actinostachys pennula</i> (Swartz) Hooker	Ray spiked fern	Florida, W Indies, C & S America	Florida endangered, related
SELAGINELLACEAE			
<i>Selaginella pallescens</i> Spring	Peacock fern	Mexico, C & S America	Horticultural, representative
<i>Selaginella uncinata</i> (Desvaux ex Poir.) Spring	Blue spike-moss	China, naturalized Florida	Horticultural, representative
THELYPTERIDACEAE			
<i>Thelypteris kunthii</i> (Desvaux) Morton	Southern shield fern	Florida, W Indies, C & S America	Representative
TAXODIACEAE			
<i>Taxodium distichum</i> (L.) Richard	Bald-cypress	Florida, SE USA	Ecological dominant
POACEAE			
<i>Oryza sativa</i> L.	Rice	Tropical Asia	Crop
<i>Saccharum officinarum</i> L.	Sugarcane	Tropical Asia	Crop
RUTACEAE			
<i>Citrus sinensis</i> (L.) Osbeck Macf.	Orange	Tropical Asia	Crop

¹Species in bold type are native to Florida. Family placements follow Hoshizaki and Moran (2001) and Flora of North America Editorial Committee (1993). Nativity follows these references and Wunderlin and Hansen (2000).

²North (N), South (S), E (East), West (W), Central (C).

³Horticultural species (Horticultural), Member of a related family (Related), Representative of genus and family (Representative).